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INVESTIGATION OF MICROBES ASSOCIATED WITH THREE VEGETABLES
Talinum triangulares (Gbure), Crassalephalum crepidoides (Ebolo), Ocimum gratissium (Efinrin)

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ABSTRACT
Occurrence of some microbes was investigated in common vegetables such as *Talinum trangularea* (Gbure);*Crassocephalum crepidoides* (Ebolo); *Ocimum gratissium* (Efinrin) The vegetable used were obtained within Offa Community (Owode Market).Two bacterial species were isolated from the vegetables such as *Staphylococcus spp, Escherichia coli*. Efforts were made to identify the source of the microbes and relevant recommendations were made.

KEYWORDS: Microbes, Staphylococcus, anti oxidants vitamins, autoclave, sterilization.

INTRODUCTION
In many African homes, vegetables form major parts of food. Vegetables are often used in the preparation of soup for human consumption. In certain parts of West Africa, the leaves are often boiled before using it for soup. It was reported by Tindal (1983) that many species probably originated in the Andean region of South America and Mexico and are widely distributed throughout most tropical areas. James (1996) reported that vegetable are excellent source of minerals and vitamins. It was also asserted that vitamins supplied through the consumption of vegetables is very high and of great value, this is because vitamins C, E and A contained in vegetables are believed to defend the body against free radical and therefore, they are termed antioxidants. These vitamins are especially abundant in fruits and vegetables. According to Stone (1995) they are essential for normal functioning of the body and usually obtained from the consumption of vegetables in the year 1665.

Vegetables, according to Oria and Rafiu (1998) is a branch of horticulture relating to the production of a member of herbaceous plants or plant part.

METHODS of MEDIA PREPARATION
The types of media used are Nutrient agar and the agars are prepared as follows. 3g of Nutrients agar was weighed on the weighing balance and was dissolved in 300cm^3 of distilled water in a conical-flask. The flask was then shaken until the agar dissolved, the flask was then corked with cotton wool and an aluminum foil avoid to contamination. The prepared media were then placed in the autoclave for sterilization at the temperature of 121^{o}C for 15min.

The Petri-dishes are washed and placed invertly in the oven for sterilization; the Petri-dishes are covered with their lid in order to avoid air contamination and the Petri-dishes are then brought out of the oven, the prepared media was then pored gently into the dishes through one side of Petri-dishes that is gently opened. The dishes containing the media were then allowed to cool at room temperature in order to allow for solidification of the media.

METHOD OF PREPARATION
The stock samples were prepared by washing the vegetable in the distilled water. This was done by slicing the vegetables into a clean piece of paper and 10g of vegetables was then weighed into a conical flask and 100cm^3 of distilled water was added to it.

METHODS of INOCULATION
The work bench was swambed with acetone for sterility. The inoculating loop was sterilized by placing it in the flame for 2minutes in order to ensure thorough sterilization (burnt red) the loop was then allowed to cool, then used for inoculating the prepared sample into the Petri-dishes containing the solidified nutrient-agar. This nutrient agar allowed the growth of the bacteria.

After each inoculating, the loop was sterilized by burning it red before using it for another inoculating and all this were done in the inoculating room also in the presence of flame in order to avoid air contamination of the media. The streaked dishes were then incubated at the temperature of 37^{o}C for 24.

BACTERIAL CELL IDENTIFICATION TECHNIQUES

There are so many techniques for bacteria identification, but the techniques used in this project are:

(a) gram staining test
(b) morphological observation

METHOD OF STAINING

This was done by making a thin smear of the cell suspension on a clean glass slide and fixed with chemical fixative that is by passing the slice containing the smear over blue flame, then the slide was flooded with crystal violet for 30-60s, then it was flooded with dilute solution of iodine, which decreases the solubility of purple dye. After that it was rinsed off in gentle tap water, then 95% of alcohol was applied which readily removes purple dye-iodine complexes from bacterial, it was flooded with red counter stain that is straining for 30-60s, it was rinsed off in gentle tap water and blotted dry with blotting paper, it was finally examined under oil immersion lens.

CALATALASE TEST

With the aid of sterile loop, a drop of Hydrogen peroxide was put on the sterile slide and loopful of the culture (organism) was picked and emulsified.

Effervescence was absent which indicates catalase negative *Escherishai coli*.

MOTILITY TEST

An innoculum of the test organism that is Staphyloccus spp was introduced into 2mls of peptone water in the test tube bottle then incubated at temperature of 37^{o}C for 24h then, the test tube was shaken thoroughly for uniform distribution of the test organism in the tube. Also one drop of it was placed on the slide free of grease with the aid of a Pasteur pipette and a cover slide was gently placed on it.

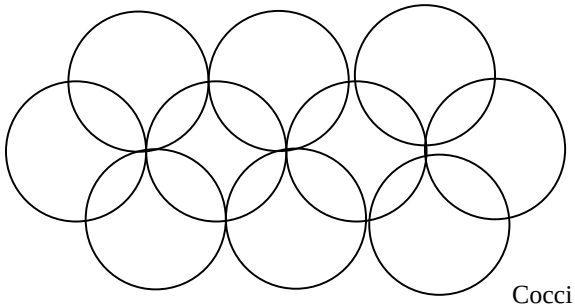
Finally, it was observed under the microscope at magnification of X40, in which there was no motility observed.

This indicated that presence of staphylococcus spp because is a non motile gram positive organism.

RESULTS

The organism listed was isolated from the vegetable under which they were listed.

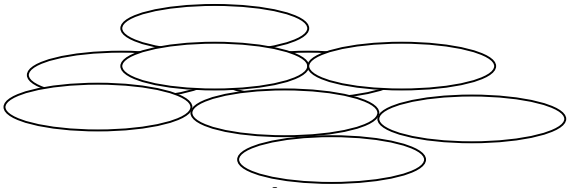
Talinum traiangularea *Staphylococci Spp*



Mg 4000 Cocci in clusters

Fig 1: *Staphylococci spp* present in Talinum Triangalanes.

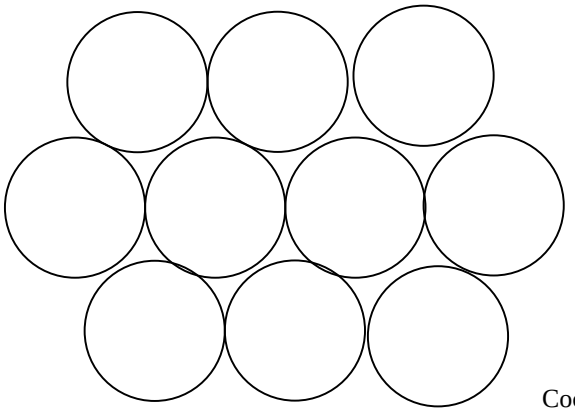
Crassocephalum crepidoides *Escherichia Coli*



Mg x 4000 Red Live Bacteria

Fig: 2 *Escherichia coli* present in crassocephalum crepidoides

Ocimum gratissium *Staphylococci Spp*



Mg 4,000 Cocci in cluster

Fig 3: *Staphylococci spp* present in ocimumgratissium

Table 1: GRAM TEST TABLE

Test	Observation	Inference
A thin smear of the cell suspension was make on a clean glass side and it was fix by passing the slide containing the smear over lue flame, then flood with crystal violet for 30-60 seconds, it was then flood with dilute solution of iodine, and it was rinsed with gentle tap water, 95% alcohol was added, then it was flooded with red counter (safranin) for 30-60 seconds then it was rinsed with gentle tap water and finally examine under oil immersion lens	Purple background observed for gram reaction	Gram positive bacteria
Specimen B		
A thin smear of the cell suspension was make on a clean glass slide and it was fix by passing the slide containing the smear over blue flame then flood with crystal violet for 30-60 seconds. It was then flood with dilute solution of iodine, and it was rinsed with gentle tap water, 95% alcohol was added, then it was flooded with red counter (safranin) for 30-60 seconds then it was rinsed with gentle tap water and finally examines under oil immersion lens	Red background observed for gram reaction	Gram negative bacteria
Specimen C		
A thin smear of the cell suspension was make on a clean glass slide and it was fix by passing the slide containing the smear over blue flame, then flood with dilute solution of iodine and it was rinsed with gentle tap water, 95% alcohol was added then it was flooded with red counter (Safranin) for 30-60 seconds then it was rinsed with gentle tap water and finally examine under oil immersion lens	Purple background observed for gram reaction	

MOTILITY TEST
Motility test showed that
Specimen A is *Staphylococcus spp*
Specimen B is *Escherichia coli*
Specimen C *Staphylococcus spp*

CATALASE TEST
From the Catalase test
Specimen A is Catalase Positive
Specimen B is Catalase Negative
Specimen C is Catalase Positive

DISCUSSION AND RECOMMENDATION
It was discovered that the dungs of the animals that has been infected by these microbes which are used in the planting of these vegetable contribute mainly to the contamination of the vegetables. The dump of the animals which serves as manure for the former serves as sources of the microbes on the vegetables. It was also discovered that most the river water used in irrigating the vegetables beds contain most of these microbes as a result of the human waste that has been deposited in them. Microbes that are also present in the soil also contribute to the contamination of the vegetables.

It was also found that improper care of the vegetables in the market causes the presence of some of these microbes on them.

The vegetables were just left on bear ground by the road side which is very unhygienic for human consumption.
Staphylococcus spp is one of the microbes that is causing vegetable contamination. Ketchum (1998) said that most microbial infection is caused by *Staphylococcus spp*. He also made the following discoveries:

- That disease cause by *Staphylococcus spp* are localized pyogenic infection of the skin such as boil and wound infection.
- It causes systemic infection that can lead to pneumonia.
- Toxin is produced while the microbes are growing on the vegetable.

James 1996 asserted that *Staphylococcus spp* are present in the nasal cavity of an infected man as well as on the skin of the animal that feed on contaminated vegetables.

Watson and Jewar (1993) reported that *Diplococcus spp* causes variety of infective disease. It is a causative agent of melioodisis disease of man. *Bacillus spp* is another microbe found on the vegetable.

Ketchum (1998) reported that it persists for a long time in contaminated field of vegetable and man consume that toxins produced by microbes on the vegetables if not properly sterile.

RECOMMENDATION
With these advantages; vegetables have gotten many African homes by using them in the preparation of soup for human consumption, I hereby recommend that there should be enough provision for disposal of waste such as public toilet by the government instead of using them as marine for growing vegetables. The government should also make enough provision for the supply of fertilizers to the farmers.

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